

## Avian Influenza Infections in Humans and Poultry of Lebanon: A Mini Review

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Received date: 07 Nov 2016; Accepted date: 09 Dec 2016; Published date: 15 Dec 2016.

Citation: Murtada M, Barbour E, Shaib H (2016) Avian Influenza Infections in Humans and Poultry of Lebanon: A Mini Review. J Emerg Dis Virol 3(1): doi <http://dx.doi.org/10.16966/2473-1846.125>

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### Abstract

Avian Influenza (AI), commonly known as “bird flu” is a viral disease that affects birds with usually asymptomatic signs in wild birds and waterfowls. In poultry, avian influenza causes high economic losses and was previously referred to as “fowl plague” in the first reported outbreak in 1878 in Italy. AI is usually host specific affecting mostly gallinaceous species. However, interspecies transmission from birds to humans was first documented in 1997 in Hong Kong leading to 18 human infections with highly pathogenic avian influenza (HPAI) H5N1 and 6 deaths caused by human exposure to poultry in live bird markets. Since then, not only HPAI H5N1 became of major public health significance causing most of the reported AI human illnesses and deaths, but also low pathogenic H5N1 and other subtypes, including H9N2 and H7 that are highly mutagenic and can convert into HPAI strains in a relatively short period of time.

Lebanon is a Mediterranean country located between major migratory bird flyways with a large population of domestic and wild bird species. Consequently, repeated AI outbreaks in poultry were recorded and documented in this country as the outbreak of 2006. This paper reviews previous reports on LPAI in poultry (H9 and H7) and humans (H9, H4 and H11) and includes the first HPAI H5N1 outbreak in domestic poultry. The potential of local AI viruses to evolve into highly pathogenic and/or zoonotic is discussed. Prevention and preparedness practices to confront the AI issue, using One Health approach is addressed.

**Keywords:** Avian influenza; Pathogenicity; Zoonosis

### AI in Poultry

Avian influenza disease that affects bird species is caused by viruses belonging to the family *Orthomyxoviridae* and genus *Influenza virus A* [1]. Avian influenza A viruses are known to be host specific infecting mainly wild and domestic bird species. In addition, HPAI H5N1 infections were reported in the early 2000s in leopards, tigers, domestic cats, dogs, stone martins, civets, and domestic pigs that were exposed to infected birds, without becoming endemic in these species [2-6]. Influenza A viruses has antigenically related matrix and nucleocapsid proteins, however are subtyped based on their hemagglutinin (H) and neuraminidase (N) proteins. Eighteen haemagglutinin (H1-H18) and 11 neuraminidase (N1-N9) have been recognized [7]. Each type is recognized by its own combination of one H and one N antigen [1]. Avian Influenza viruses are divided into two groups according to the virulence of the strain and its ability to cause high mortality in poultry. The first group is highly pathogenic avian influenza (HPAI) that is known to cause flock mortality of up to 100% [1]. H5, H7 and H9 are the only subtypes causing HPAI to date [8]. Nonetheless, not all of these subtypes cause HPAI; many cause milder disease in poultry and are grouped under Low Pathogenic Avian Influenza (LPAI). HPAI and LPAI H5/H7 that might become highly pathogenic by mutation are both on the OIE list of notifiable diseases [8]. AI was first reported as “Fowl Plague” referring to highly pathogenic avian influenza (HPAI) in 1878 by Perroncito in Italy [9]. Economic losses from AI are mainly caused by HPAI that causes severe mortality in domestic birds. Additional inputs result from HPAI outbreaks, including the cost of: disposal, depopulation, cleaning, disinfection, quarantine, and surveillance cost, and losses from high mortality and morbidity and indemnities paid for birds [10]. However, indirect costs such as losses in poultry exports, farmer’s loss of income, and decrease in consumer demand for poultry

can increase losses by 5-10 folds. For instance, significant economic losses were caused by HPAI outbreaks in United States from December 2015 through July 2015. During these outbreaks 48 billion birds mostly laying hens (38.4 million) were depopulated at an estimated direct cost of \$1.6 billion with economy-wide impact estimated at \$3.3 billion [11]. Losses from LPAI are mostly due to a secondary infection in birds and include mortality, carcass condemnations, secondary bacterial medications, cleaning and disinfection and delayed placement of new flocks [10]. LP AI endemics such as H9N2 poultry infection in Asia and the Middle East in the late 1990s and early 2000s, and H5N2 in Mexico, in the 1990s, have caused great economic losses that were poorly reported [10].

### AI in Lebanon

Lebanon is a Mediterranean country located in the Middle East, Asia, surrounded by countries that have reported poultry and human infection with HPAI H5. Moreover, Lebanon lies between 2 important wild bird migratory flyways, the Black Sea/Mediterranean flyway and the East Africa/West Asia flyway [12]. Lebanon has a high domestic poultry population of about 80.8 million birds divided into 77 million broilers and 3.8 million laying hens [13]. Lebanon also neighbors Syria and currently witnesses increased illegal movement of domestic animals from Syria to Lebanon due to the current War situation in Syria and the uncontrolled boundaries. All of these facts increase the risks of AI spread in Lebanese poultry and human populations.

### LPAI in Lebanese poultry

Low pathogenic avian influenza (LPAI) in Lebanon was first reported as H9N2 in 2006 by Barbour et al [14]. This outbreak caused significant decrease in egg production of 46% in broiler breeder and 47.3% in laying

hen farms. Flocks were confirmed for AI using ELISA and then random ELISA-positive samples revealed H9 specific antibodies by HI test. The virus was further subtyped as H9N2 by Central Veterinary Laboratory in Weybridge, UK. Reporting of H9N2 was not limited to poultry; specific H9 antibodies were also found in pigs eating infected poultry at 100% [14]. The second infection of Influenza type A was reported by the same researcher in 2007. In a collaborative work between the Lebanese Ministry of Agriculture and the American University of Beirut, a surveillance of type A influenza viruses was conducted in migratory wild birds, resident wild birds, pet birds and farm birds, using the reverse transcription-polymerase chain reaction (RT-PCR) technique. Results showed that 14.3% of the collected samples were positive for AI. All positive samples underwent RT-PCR subtyping for H5 and H7 genes and were negative for H5. Only 6.8% of the AI positive samples were found to be of the LPAI H7 type. The H7 positive samples were restricted only to sparrows (resident wild bird species) and to backyard chicken in the south province of Lebanon [15].

### **Pathogenicity and oseltamivir resistance of LPAI isolated from broilers in Lebanon**

Although losses from LPAI viruses are lower, compared to those resulting from HPAI; H9N2 LPAI viruses in Lebanon demonstrated an ability to increase their pathogenicity, and host range, following their passing in different hosts. A study was conducted to evaluate the effect of nasal viral passing of Lebanese isolate of H9N2 AI virus in hamsters on its interspecies-pathogenic adaptability by monitoring the variation in amino acid sequences of hemagglutinin (HA) and neuraminidase (NA) proteins [16]. Results showed that there was 100% similarity in amino acid sequence of HA gene in most passaged viruses. The amino acid sequence of the neuraminidase gene showed an antigenic drift caused by a R46P point mutation that might explain the pathogenic adaptability of the third passage viruses in hamsters' lungs [16]. The isolated R46P H9N2 virus showed resistance to oseltamivir in comparison with other three H9N2 viruses that were susceptible at a percentage ranging between 63-100% [17]. These results confirm the World Health Organization's alertness for possible zoonotic potential of adaptable H9N2 [16]. Another similar study was done on broilers by multiple passages of H9N2. This work resulted in conserved dibasic R-S-S-R amino acid sequence of the viral hemagglutinin cleavage site and a variability in the neuraminidase amino acid sequence. These changes were also associated with higher pathogenicity in the third and final passage H9N2 viruses that was apparent by increased morbidity [18]. A third study of multiple passages of LPAI H9N2 was conducted in chicken embryos. The mortality among chicken embryos jumped from 0% in passage 0 original H9N2 virus to 86.7% and 100% of second and third H9N2 passages respectively [19]. Results indicate that antigenic drift caused by point mutations of the neuraminidase viral proteins occur under selective pressure of immune responses. These point mutations prove that in addition to antigenic shifts caused by viral reassortment, antigenic drifts can play a role in the increased pathogenicity and persistence of LPAI viruses in population [20].

### **HPAI H5N1 in Lebanon**

Recently, on the 23<sup>rd</sup> of April 2016 Lebanon officially reported, for the first time, HPAI in domestic poultry farm in the village of Nabichit in Bekaa region located near the Lebanese-Syrian border [21]. The owner of the farm reported abnormal flock mortality reaching up to 100%. The infected chicken farm and other surrounding farms were immediately zoned and put under quarantine. Twenty thousand birds were dead due to HPAI infection and 60000 were stamped out [21]. Three weeks later, the second outbreak of HPAI H5N1 was reported to the OIE on 14<sup>th</sup> of June 2016 in a nearby chicken layers farm located in Sariin Tehta in the protection zone around the primary reported outbreak [22]. In the second outbreak 20000 birds died from HPAI infection and 106000 birds

in nearby farms were stamped out. The source of the two outbreaks was reported to be illegal animal movement from Syria to Lebanon [21,22] that is currently increasing due to the war situation in Syria and uncontrolled boundaries between the two countries. HPAI H5N1 outbreaks in Lebanon caused a great public health concern about the possibility of HPAI transmission to humans through the consumption of poultry eggs and meat. Consequently, chicken live weight prices and prices of eggs reached the lowest recorded decrease in 2016 reflecting the economic impact of H5N1 on the poultry sector.

### **AI in Humans**

Interspecies transmission of type A AI from birds to humans was firstly documented in 1997 in Hong Kong leading to 18 human infections with highly pathogenic avian influenza (HPAI) H5N1 and 6 deaths caused by human exposure to birds [23].

Since then, HPAI H5N1 and LPAI H7N9 were proved to be of greatest zoonotic potential becoming a major public health concern and causing most of the reported AI human illnesses and deaths [24]. Exposure to live birds has been a major risk factor associated with most of the AI human cases.

### **Factors restricting the host range of avian influenza A Viruses**

Influenza viruses belonging to the *Orthomyxoviridae* family are divided into A, B and C based on group specific antigens. Only influenza type A viruses that include avian and swine influenza viruses are of zoonotic importance [25]. AI viruses are known to infect mostly avian species and rarely humans unlike the human H1N1 and H3N2 influenzas that occur each year [26]. Differences in influenza transmission and replication may arise from the variation in cell receptor specificity in the respiratory tract epithelium of the host. Avian influenza viruses bind preferably to N-acetylneuraminic acid- $\alpha$ 2,3-galactose linkage on sial-oligosaccharide ( $\alpha$ 2,3 linkage) receptors, while human influenza viruses bind preferably to N-acetylneuraminic acid- $\alpha$ 2,6-galactose linkage on sial-oligosaccharide ( $\alpha$ 2,6 linkage) receptors. Avian respiratory epithelium has predominant  $\alpha$ 2,3 linkage but human respiratory epithelium has predominant  $\alpha$ 2,6 linkage. Although the human respiratory tract does have  $\alpha$ 2,3 receptors in cells deep in the respiratory system, the location of these receptors make human AI viral infections infrequent [27]. Moreover, the AI polymerase complex and other viral genes may be also responsible for inefficient replication in humans [27].

The second factor that plays an important role in restricting the host range of AI is the proteolysis of the hemagglutinin that is done by cellular proteases in the HA cleavage site to make the virus infectious. Mammalian influenza viruses have arginine amino acid that marks the HA cleavage site. Proteases able to split this site are only found in epithelial cells of mammalian respiratory system making mammalian influenza infection restricted to the respiratory system [20]. However, avian influenza viruses have multi-basis amino acids on the cleavage site making AI infection in birds a systemic infection since all body organs of birds contain the ubiquitous proteases that are able to bind and split the cleavage site. Moreover, bacterial proteases from *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Klebsiella* spp., are able to split the HA cleavage site and activate the virus causing human infection with AI [28].

### **Avian influenza virus infection in humans**

H5N1 was the first avian influenza virus reported to infect humans in 1997. A global epidemiological study showed that there were 907 H5N1 human cases between May 1997 and April 2015 of whom 483 case fatalities occurred 53.5% yielding H5N1 to impose the highest zoonotic threat [29]. The second type of AI viruses isolated from humans was H9N2 [30]. Both H5 and H9 AI viruses were genetically linked to AI found in quails [20]. More subtypes of AI in humans were isolated from humans, namely

H7N7 and H7N9 [31,32]. Avian influenza subtype H7N7 was isolated in an epizootic in the Netherlands in 2003. Several people were infected and one case fatality was reported during the 2003 H7N7 outbreak [31]. In 2013, a new AI subtype, namely H7N9 virus was isolated from human patients in China. The H7N9 LPAI caused 440 human infections with at least 155 deaths from the disease showing symptoms of high fever, bronchitis, pneumonia, and dyspnea [32].

### LPAI human infections and the risk of HPAI H5N1 human infection in Lebanon

In Lebanon, reported AI human infections belong to LPAI group. Lebanese scholars found that 32.3% of individuals exposed to H9 infected poultry had elevated antibody titer against viruses of the same subtypes [14]. Using serological tests, Kayali et al. [12] showed a cross-sectional evidence of human infection with H4 and H11 avian influenza viruses among Lebanese chicken growers. Although no symptoms have been observed among farmers, this study indicated that H4 and H11 LPAI can be of zoonotic importance. Moreover, human exposure to live birds was still recognized as a major risk factor in AI human infections. Although, recently, Lebanon reported HPAI in domestic poultry [21,22], human infection with HPAI is still not evident in this country. Epizootic HPAI H5N1 in Lebanon caused a great public health concern about the possibility of HPAI transmission to humans through the consumption of poultry eggs and meat. Literature review shows that AI human infections were mostly restricted to exposure to live poultry and not to consumption of poultry products, with no evidence of human-to-human transmission [33]. In Lebanon feeding dead birds to swine is a common practice [15]. This fact adds up to the risk of human infection with HPAI since pigs might act as a reassortment vessel when infected with both human and avian influenza viruses rendering HPAI adaptable for human transmission. A recent research reported that humans could also be a "mixing vessel" when dually infected with human and avian influenza viruses at the same time [34]. This means that poultry farmers and workers in Lebanon are at a high risk of human H5N1 infections after the first reported HPAI H5N1 in poultry. Table 1 summarizes the confirmed human and poultry infection with AI viruses in Lebanon from 2006 to present.

### Conclusion and Recommendation

Both types of avian influenza viruses (LPAI and HPAI) are circulating in Lebanon. Low Pathogenic avian influenza viruses were detected in both poultry and farmers. So far, highly pathogenic avian influenza viruses were only reported in poultry. Regarding HPAI, Lebanon might witness sporadic outbreaks in poultry leading to economic losses and public health concerns. In addition, Lebanese LPAI viruses pose serious threat

due to the fact that these viruses can mutate into HPAI type, widen their host range, besides their resistance characteristics to anti-influenza drugs, namely oseltamivir.

The Government of Lebanon should conduct continuous surveillance and not only sporadic targeted surveillance to monitor HPAI in poultry, high risk individuals exposed to poultry and wild birds. Moreover, the Government of Lebanon should appoint a team having One Health Approach to tackle this risk and put a preparedness plan for any future human infection with H5N1 in Lebanon. Research should backup governmental efforts in controlling AI outbreaks, specifically in developing innovative vaccines for poultry.

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Year	Subtype	Patho type	Host species	Affected	Reference
2006	H9N2	LPAI	Humans	11	[14]
2006	H9N2	LPAI	Pigs	3	[14]
2006	H9N2	LPAI	Domestic Poultry	24 farms	[14]
2007	AI type/ not typed	LPAI	Domestic Poultry	134	[15]
2007	AI type/ not typed	LPAI	Pet Birds	11	[15]
2007	AI type/ not typed	LPAI	Resident Wild Birds	15	[15]
2007	AI type/ not typed	LPAI	Migratory Birds	30	[15]
2007	H7	LPAI	Domestic Poultry	12	[15]
2007	H7	LPAI	Resident Wild Birds	1	[15]
2010	H4	LPAI	Humans	3	[12]
2010	H11	LPAI	Humans	2	[12]
2016	H5N1	HPAI	Domestic Poultry	20000	[21]
2016	H5N1	HPAI	Domestic Poultry	20000	[22]

**Table 1:** List of confirmed human and poultry infections with avian influenza viruses in Lebanon from 2006 to present

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